UNIVERSITY OF OKLAHOMA MEDICAL CENTER

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Technical Report No. 10
University of Oklahoma Medical Center THEMIS Contract

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These studies were conducted to determine the effects of dopamine on the canine liver before and following endotoxin administration. Isolated, denervated livers were perfused via arterial and venous inflow vessels at constant rates by donor dogs. Weights were continuously recorded throughout the experiments. There were marked reductions in liver weight with dopamine infusion prior to endotoxin administration and continued reductions at slower rates following endotoxin. The mechanism of liver shrinkage is postulated to be primarily due to sinusoidal contractions independent of changes in liver venous resistances. Dopamine markedly increased hepatic artery pressures, thus hepatic artery resistances increased since the flows were constant. In the donor animal, dopamine elevated systemic arterial pressure, pulse pressure, and heart rates.

Introduction

The systemic arterial hypotension following endotoxin administration in the dog has been ascribed to pooling in the liver (MacLean et al., 1956; MacLean and Weil, 1956) with subsequent decrease in venous return (Weil et al., 1956). The primary site of action of endotoxin in the liver has been postulated to be on the intrahepatic venous system with subsequent myogenic response in the hepatic artery segment (Hinshaw et al., 1966). The hepatic vein constriction results in pooling in the liver which is passively reflected by an increase in the portal vein pressure (Hinshaw et al., 1966). Parallel studies in this laboratory, utilizing a venous return preparation in which the cardiac inflow is held constant, show that a constant infusion of dopamine (3, 4-dihydroxyphenylethylamine) caused a significant reduction in the degree

of pooling following endotoxin administration in the intact dog.

The isolated perfused liver preparation has been shown to be an excellent preparation for evaluating the vascular actions, independent of neural actions, of endotoxin on the liver. There is also the additional advantage of being able to readily control the blood flows, thus pressure changes reflect resistance changes (Hinshaw et al., 1966). For these reasons, the isolated liver perfused by a donor dog was used to further study the actions of dopamine in endotoxin shock. Various parameters in the donor dog were also monitored in order to obtain an over-all picture of the actions of dopamine in endotoxin shock.

Methods

Ten experiments were carried out on isolated, weighed livers perfused by blood from a donor dog. Mongrel dogs of either sex were used (7.8 to 10.5 kg). The dogs were anesthetized with sodium pentobarbital (30 mg/kg). The preparation utilized was a modification of a technique previously described (Hinshaw et al., 1966). In order to obtain the isolated liver, a laparotomy was performed and the stomach and spleen were removed for easier access to the liver. The hepatic artery, portal vein, and common bile duct were freed up from surrounding tissue. The animal was then heparinized (3 mg/kg). The dog was bled to obtain blood from the reservoir system. The vessels for cannulation were then ligated and the liver was rapidly removed and placed in iced saline where the hepatic artery, portal vein, and common bile duct were cannulated, and a cannula was inserted into a hepatic vein. The cystic duct was ligated to negate any changes which might be due to humoral effects on the gallbladder. The common bile duct was cannulated to allow free

drainage of the bile outside of the blood reservoir system. The liver was then transferred to a strain-gauge weighing device and perfused at constant, pulsatile flow by Sigmamotor pumps. The hepatic artery was perfused by blood from the aorta of the donor dog and the portal vein was perfused by blood from the inferior vena cava of the donor dog (Figure 1). Transfer of the liver to the extracorporeal circulation required less than two minutes. Adequate perfusion was determined by the liver remaining in an isogravimetric state and the hepatic artery and portal vein pressures within physiological range (60-150 mm Hg and 3-15 mm Hg respectively). Hepatic artery flow (range 48 to 132 cc/min) usually exceeded portal vein flow (range 12 to 60 cc/min) as has been shown in previous studies (Hinshaw et al., 1966; Shoemaker, 1954). Donor dogs remained intact except for the cannulations, isolated livers having been removed from separate animals. No blood reactions were observed. Liver blood temperature was maintained constant by a water bath through which the inflow tubing passed. Only plastic materials were used in the perfusion system.

Pressures were measured by Statham pressure transducers, and recorded on a four-channel Sanborn recorder. Hepatic and portal vein pressures were alternately recorded on the same pressure transducer for greater accuracy. Liver weight changes were continuously recorded.

The experiments were divided into two groups, untreated and treated, each consisting of five experiments. The untreated group received a constant, isotonic saline infusion (3.98 cc/min) started 10 minutes prior to the endotoxin and maintained for 30 minutes post-endotoxin. The experiments were followed for an additional 10 minutes to determine whether the

infusion, per se, produced any changes in the measured parameters. The procedure for the treated groups was the same as the untreated, except that a dopamine infusion (3.98 cc/min) was given in place of the saline infusion. The E. coli endotoxin given was an established LD_{80} based on the weight of the intact dog (1.5 mg/kg). The dopamine (3,4-dihydroxyphenylethylamine, California Biochemical Corporation) was made up in 0.9 per cent saline to a concentration of 100 µg/cc. The infusion rate chosen (3.98 cc/min) has been previously established as slightly above the threshold for beneficial responses in the intact dog (in press results). This isolated liver preparation has been shown to be relatively stable over a 160-minute period (Hinshaw et al., 1966). Statistics were performed by a modification of the students "t" test. In the interpretation of the results, significance is defined as p values equal to or less than 0.05.

Results

Figure 2 presents the mean cumulative pooling values for the two groups: The large increase in liver volume after endotoxin is entirely prevented with dopamine infusion; liver volume decreasing markedly during the period of infusion ($p < 0.01$ until the dopamine infusion was stopped at 30 minutes; $p > 0.10$ at 40 minutes).

Figure 3 shows the mean values for the hepatic vein, hepatic artery, and portal vein pressures of the isolated, perfused liver for the untreated and treated groups. The values from -10 minutes to 0 time reflect the effects of the infusions only, from 0 to 30 minutes the effects of endotoxin only (untreated) as compared with endotoxin and dopamine (treated), and from 30 to 40 minutes the effects of endotoxin only in both groups. The hepatic

vein pressures were not significantly different ($p > 0.10$) throughout the course of the experiments (-10 to 40 minutes). The hepatic artery pressures were not significantly different ($p > 0.10$) during dopamine infusion (-10 to 30 minutes); however, following termination of dopamine infusion $0.01 < p < 0.05$ at 35 and 40 minutes. The portal vein pressures were significantly different ($p < 0.05$) during dopamine infusion (15 to 30 minutes) and after the dopamine infusion was stopped $P < 0.01$ at 35 and 40 minutes.

Results of the measured parameters in the donor dog in the untreated group reflect changes with endotoxin similar to those in previously reported studies (MacLean et al., 1956; MacLean and Weil, 1956; Hinshaw et al., 1964). The action of dopamine, in the treated group as illustrated in succeeding figures, shows some significant effects: Figure 4 shows that mean systemic arterial pressure is maintained in the normal range by dopamine infusion after endotoxin injection. Comparisons of pressures of untreated and treated animals show that at 0 time $p > 0.10$, from 5 to 30 minutes $p < 0.05$, and at 35 to 40 minutes $p > 0.10$. Figure 5 illustrates the pulse, systolic, and diastolic pressures with and without dopamine infusion. None of these pressures were significantly different ($p > 0.10$) pre-endotoxin (-10 to 0 minutes) and post-infusion (30 to 40 minutes). However, during dopamine infusion, $0.10 < p > 0.05$ at 5 minutes and from 10 to 30 minutes $p < 0.01$. The elevation in pulse pressure is due to the systolic pressure ($p < 0.01$ from 5 to 30 minutes) being elevated to a greater extent than diastolic pressure ($p < 0.05$ from 5 to 30 minutes). Figure 6 shows that the heart rate is significantly elevated above the untreated group with dopamine administration until the infusion is stopped.

Figure 7 tabulates the directional changes with endotoxin (untreated group 0 to 40 minutes), dopamine (reflected by post-infusion changes, 30 to 40 minutes), and the combination of dopamine and endotoxin (treated group, 0 to 30 minutes). The responses of the pressures and changes in liver blood volume to endotoxin compare well with previously reported results (Hinshaw et al., 1966). Dopamine, per se, does not have any effect on hepatic vein pressure, since there was no significant difference between the two groups throughout the experiments. There was a significant difference in the hepatic artery pressures post-infusion of dopamine showing that dopamine increased hepatic artery resistance. Since portal vein pressures are passively associated with the effects of changes in sinusoidal blood volume, and dopamine markedly decreased liver blood volume, it had no direct action on portal vein pressure. Comparing the combined effects of dopamine and endotoxin (III) with the effects of each administered individually (I and II), it is seen that the increase in the hepatic vein pressure in III is due entirely to endotoxin. The increase in the hepatic artery pressure is due primarily to endotoxin but there is some contribution from dopamine (no significant difference between I and III until the dopamine infusion was stopped). There is an increase in portal vein pressure in III due to a decrease in the rate of release of blood from the liver after endotoxin. In III, dopamine caused a certain rate of release of blood from the liver which decreased when endotoxin was given.

Discussion

These studies show that dopamine has a direct action on the liver, for the responses were removed as soon as the dopamine infusion was stopped

(within 20 seconds). Dopamine caused a release of blood from the liver, even after endotoxin was given. The release of blood after endotoxin was not as great as before endotoxin. These changes in liver blood volume with dopamine were so great that they could be observed visually. When dopamine infusion was stopped, the degree of pooling reached that of the untreated group within ten minutes.

Previous observations have suggested that pooling in the liver after endotoxin is the initial response leading to systemic hypotension in the dog (MacLean et al., 1956; MacLean and Weil, 1956). Weight changes in the isolated perfused liver are similar to those of the "in situ" liver (MacLean et al., 1956; MacLean and Weil, 1956: Hinshaw et al., 1964). Early vascular responses of the isolated liver to endotoxin are not due to release of agents from extra-hepatic sites since responses of the liver to perfusion by a heart-lung preparation or by a donor dog are indistinguishable (Hinshaw et al., 1966). Responses of the liver are not dependent on an innervated splanchnic bed or secretion from the adrenal glands (Hinshaw et al., 1964). The possibility has been suggested by Hinshaw et al. (1966) that endotoxin may exert a direct effect on hepatic vessels, since no vasoactive agent or combination of agents produce the same liver responses to endotoxin. Others have reported similar observations (Jacobson et al., 1964).

Results from these studies with endotoxin confirm the above mentioned results. The isolated liver pressure responses were not quite as great in this study, which is probably due to the endotoxin being given to the donor dog; whereas, in the previous studies by Hinshaw et al. (1966), the endotoxin was given to the isolated perfused liver. Parallel studies in this laboratory have shown, that with the venous return preparation in which the cardiac inflow

is held constant, dopamine prevents pooling in the dog. At the threshold concentration of dopamine used, there is a systemic pressor response in the dog. Dopamine infusion maintained systemic arterial pressure, pulse pressure and heart rate following endotoxin administration in the donor dog until the infusion was stopped. These results confirm other observations from this laboratory and those of other investigators (Maxwell et al., 1960; Goldberg and Sjoerdsma, 1959; Black and Rolett, 1966). Regarding the mechanism of action of dopamine on the liver, since the hepatic arterial and portal inflows were held constant and there was no significant difference in the hepatic vein pressures of the untreated and treated groups, there exists the possibility that dopamine produces active contraction of the sinusoidal linings of the liver. Increased sinusoidal permeability could account for pooling of blood in the liver by increasing the amount of fluid in extravascular compartments. However, it is difficult to account for the marked release of blood from the liver with dopamine, both before and following endotoxin administration, by any mechanism other than contraction of the sinusoidal linings.

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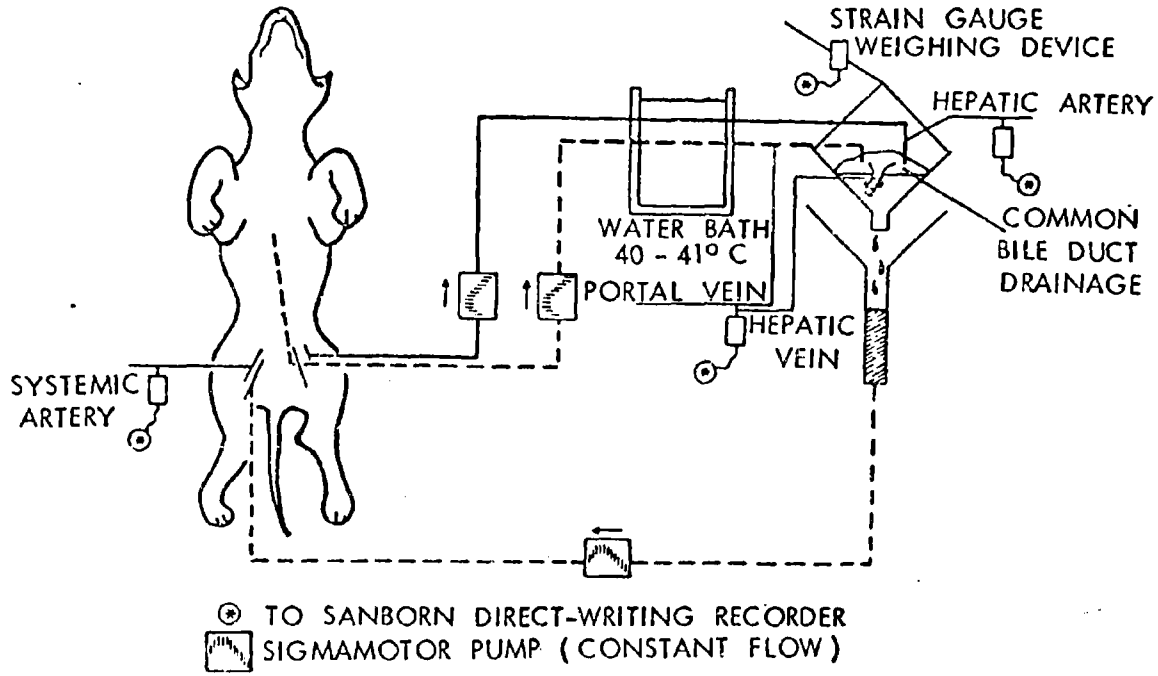

Figure 1. Dog-perfused liver preparation

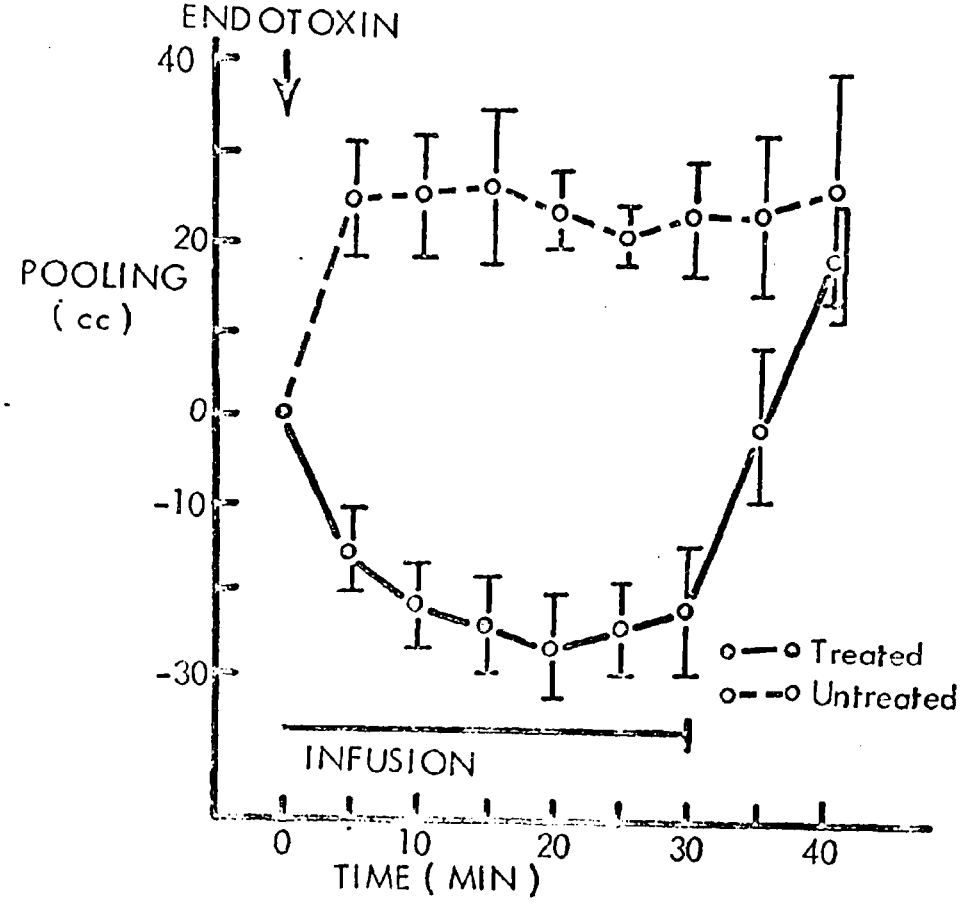

Figure 2. Effect of dopamine infusion on liver pooling after endotoxin in the isolated perfused liver. Points represent means ± S.E. Untreated: endotoxin with saline infusion; Treated: dopamine infusion with endotoxin injection.

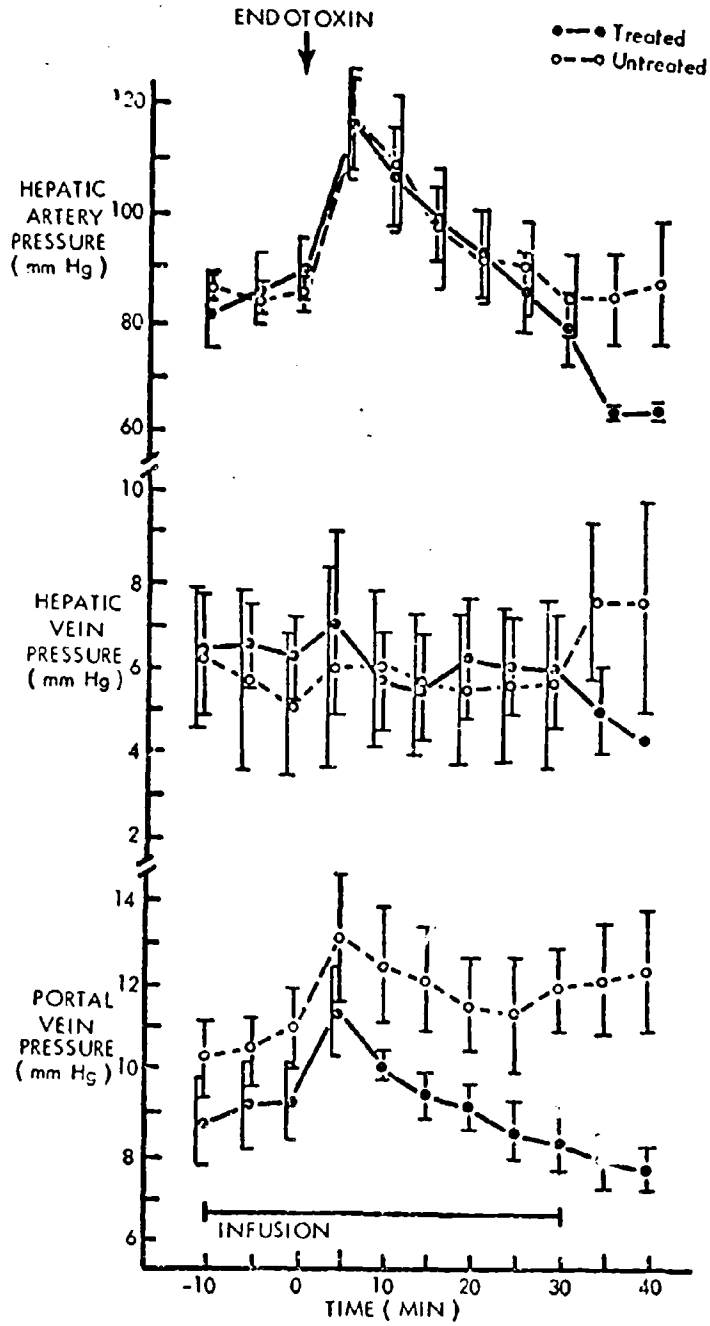

Figure 3. Effect of dopamine infusion on hepatic vein, hepatic artery, and portal vein pressures after endotoxin in the isolated perfused liver. Points represent means ± S.E. Untreated: endotoxin with saline infusion; Treated: dopamine infusion with endotoxin injection.

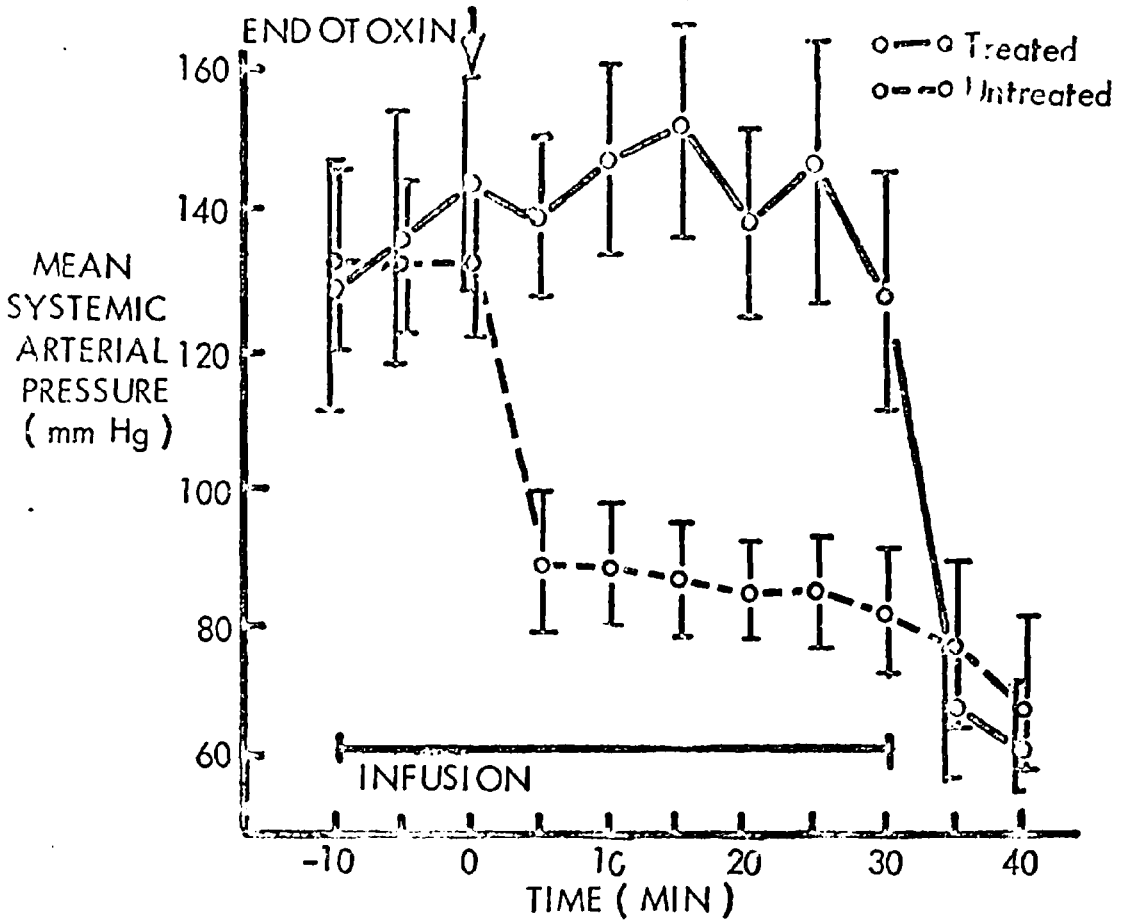

Figure 4. Effect of dopamine infusion of mean systemic arterial pressure after endotoxin in the donor dog. Points represent means ± S.E. Untreated: endotoxin with saline infusion; Treated: Dopamine infusion with endotoxin injection.

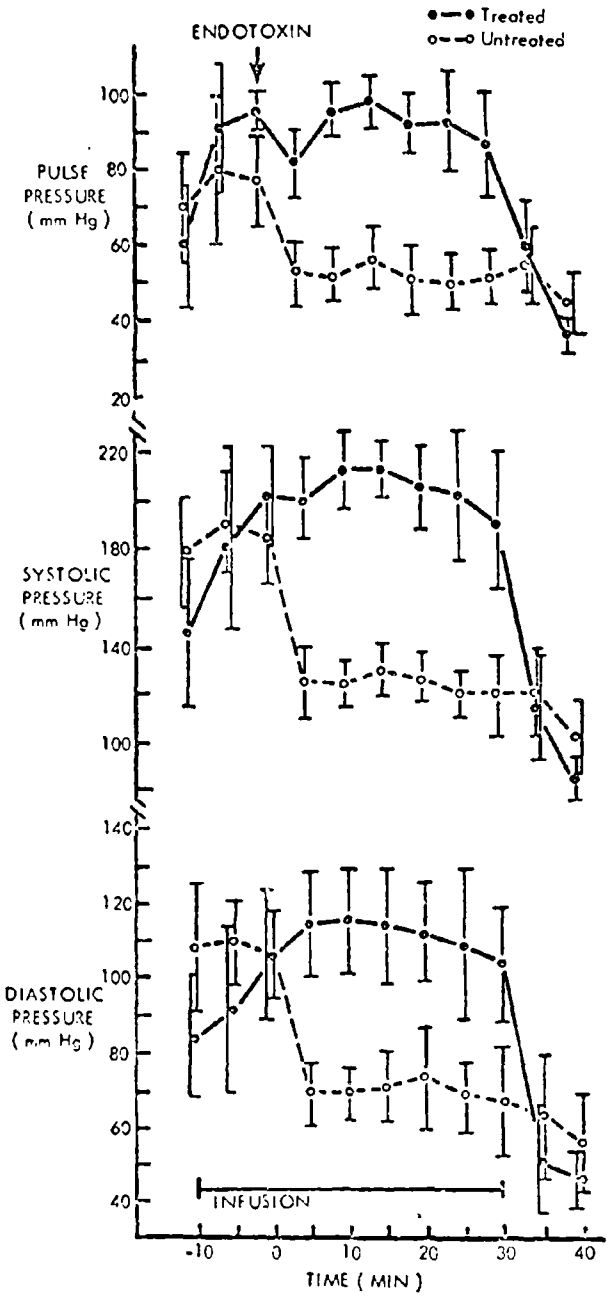

Figure 5. Effect of dopamine infusion on pulse, systolic, and diastolic pressures after endotoxin in the donor dog. Points represent means ± S.E. Untreated: endotoxin with saline infusion; Treated: dopamine infusion with endotoxin injection.

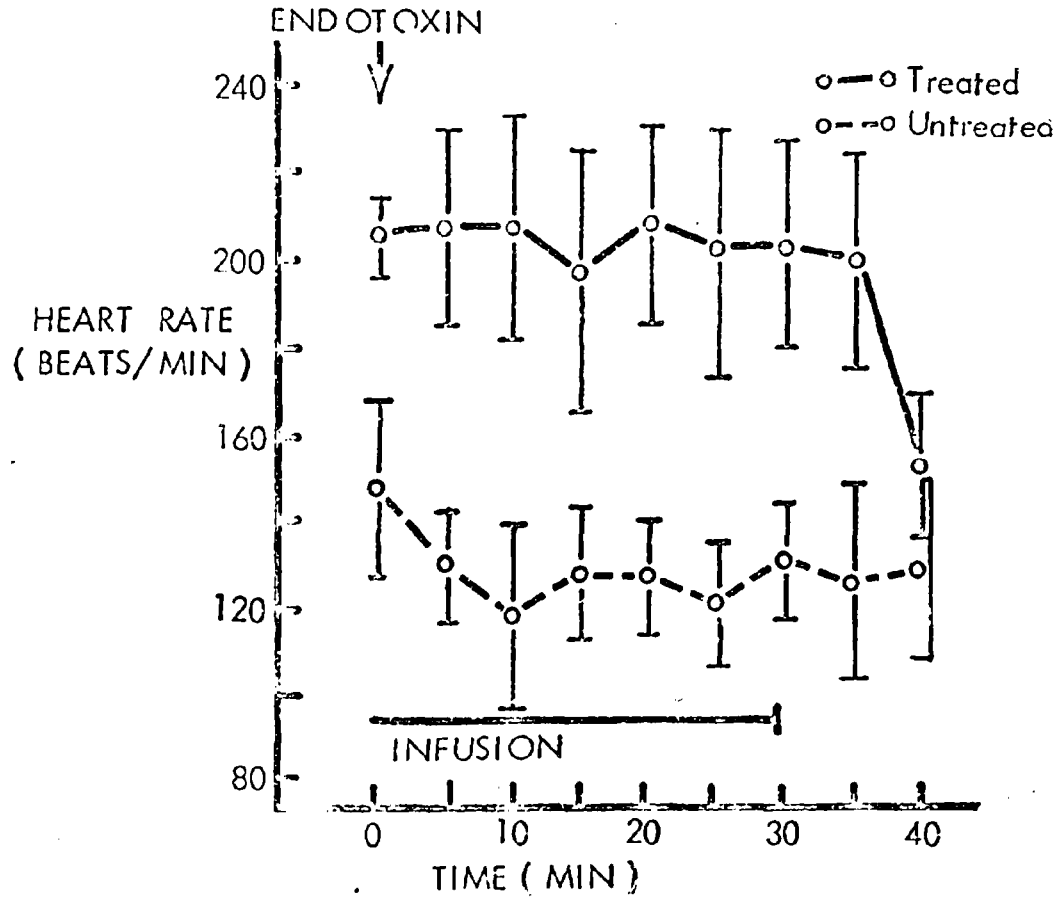

Figure 6. Effect of dopamine infusion on heart rate after endotoxin in the donor dog. Points represent means ± S.E. Untreated: endotoxin with saline infusion; Treated: dopamine infusion with endotoxin injection.

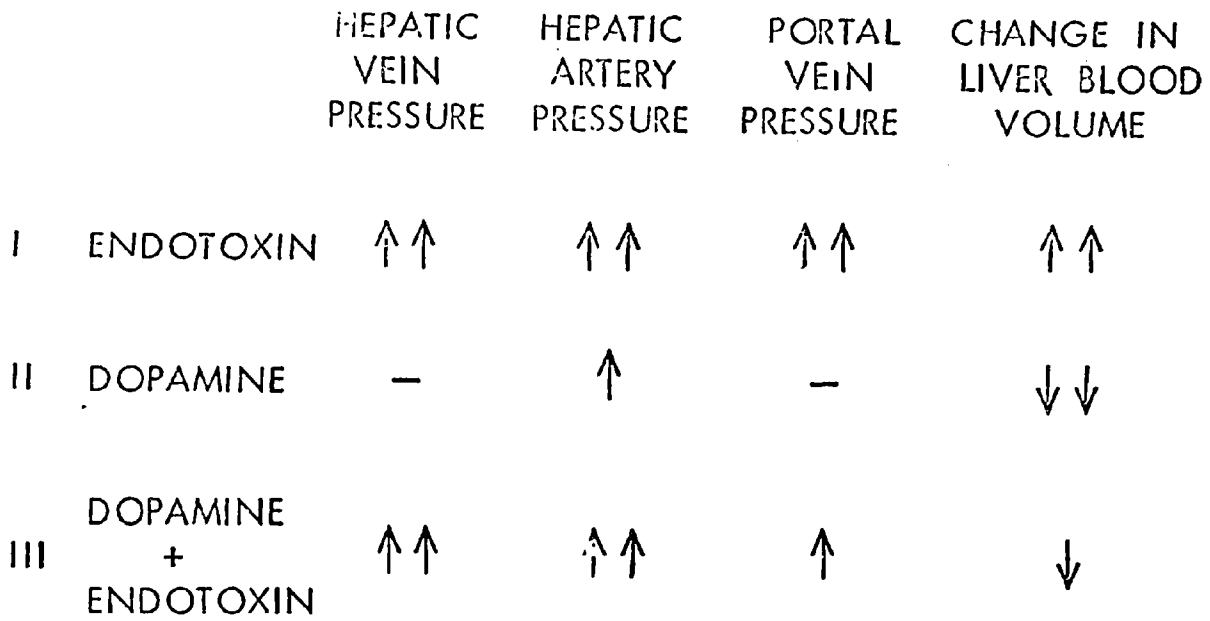

Figure 7. Comparative actions of dopamine and endotoxin on the isolated perfused liver.

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13. ABSTRACT

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